rectified in accordance with the present amendment, thereby rendering these grounds of objection moot.

Applicants acknowledge the requirement for filing a certified copy of the British priority application under 35 U.S.C. §119(b) and are in the process of obtaining a copy of the same. This document will be forwarded to the United States Patent and Trademark Office upon receipt in the office of the undersigned.

An abstract of the disclosure is provided to satisfy the requirements for the same as set forth by the Examiner at page 3 of the Official Action.

Claims 1-2, 5-11, 16, and 17 have been rejected under 35 U.S.C. §112, second paragraph as allegedly indefinite for failing to particularly point out and distinctly claim the subject matter regarded as the invention.

At page 5 of the Official Action, the Examiner has rejected claims 1, 2, 5-17, 21, 26-29 and 32 under 35 U.S.C. §103(a) as allegedly obvious over Waterhouse et al. taken with Wassenegger et al.

Applicants respectfully submit that the claims as presently amended are in condition for allowance. Each of the above-noted objections and rejections under 35 U.S.C. §112, second paragraph and §103 is, therefore, respectfully traversed.

CLAIM 1 AS AMENDED AND CLAIMS DEPENDENT THEREFROM FULLY SATISFY THE REQUIREMENTS OF 35 U.S.C. §112, SECOND PARAGRAPH

Claims 1, 2, 5-11, 16 and 17 are allegedly indefinite for the recitation of the term "approximately". This term has been omitted from the claims in order to advance prosecution of the present application. Specifically, the claims have been amended to recite "short RNA molecules which are 21-25 nucleotides in length". Support for the present amendment can be found at page

4, lines 4-14. The present amendment renders the claims in full compliance with 35 U.S.C. §112, second paragraph. Accordingly, Applicants request that the rejection of the claims on this basis be withdrawn.

THE COMBINED DISCLOSURES OF WATERHOUSE ET AL. AND WASSENEGGER ET AL. FAIL TO RENDER THE SUBJECT MATTER CLAIMED IN CLAIMS 1,2 5-17, 21, 26-29 AND 32 OBVIOUS

The Examiner has rejected claims 1, 2, 5-17, 21, 26-29 and 32 as allegedly obvious over the combined disclosures of Waterhouse et al. and Wassenegger et al. The Examiner asserts that Waterhouse et al. teach post-transcriptional gene silencing using constructs that form double-stranded mRNA. The Examiner further asserts that Wassenegger et al. teach that a 43 nucleotide long element forms a double stranded RNA molecule which is subsequently degraded as well as that PTGS homology of 60-130 or 10-100 base pairs between an inactivating transgene and the target sequence can lead to PTGS. Based on these assertions, the Examiner concludes that it would have been obvious to one of skill in the art to modify the teachings of Waterhouse et al. by identifying small RNA molecules that can silence a gene with a reasonable expectation of success. The Examiner further asserts that one of skill in the art would have been motivated to make such a modification given the combined teachings of Waterhouse et al. and Wassenegger et al.

The criterion for determining obviousness under §103 is whether the prior art supplies some motivation or incentive to one of ordinary skill in the art to arrive at the invention as claimed. In re Dow Chemical Company, 5 U.S.P.Q. 2d 1929 (Fed. Cir. 1988). Obviousness cannot be established by combining teachings of the prior art to produce the claimed invention, absent some teaching or suggestion supporting the combination.

In re Fine, 5 U.S.P.Q.2d (Fed. Cir. 1988). Moreover, the teaching or suggestion supporting the desirability or the combination must be found in the prior art, not in applicant's disclosure. In re Fritch, 23 U.S.P.Q.2d 1780 (Fed. Cir. 1992). Under these standards, neither of the cited references, considered singly or in combination, render obvious the invention as claimed in claims 1,2 5-17, 21, 26-29 and 32.

The instant application teaches that SRMS are exclusively detectable in organisms that exhibit PTGS (page 2). "SRMS" are defined collectively as nucleic acids which encompass short antisense RNA molecules (SARMs) and short sense RNA molecules (SSRMs).

The SRMs are about 25 nt in length (e.g. 25 ± 5 nucleotides). The specification teaches methods for isolation of these SRMs enabling production of efficient antisense reagents for use in the modulation of the PTGS machinery in targeted organisms (page 9, lines 26 - 30).

As the Examiner correctly points out, Waterhouse et al. do not teach methods for screening and isolation of short RNA molecules of about 25 nucleotides in length which silence target genes. Waterhouse et al. simply teach that when the plant genome comprises artificially introduced sense and antisense genes co-located therein, or a single transcript that has self-complementarity, or a sense and antisense transcript brought together through crossing, such sequences may confer virus immunity or gene silencing in the plant (see abstract). Waterhouse et al. then propose a model that is consistent with their findings and those of others. At no point do Waterhouse et al. describe or suggest that their findings are due to an RNA species of a certain minimal length and neither do they suggest methods for design of short RNAs made to "fit" into the PTGS machinery. The only size of any RNA mentioned is of a 558 bp mRNA

generated from a truncated GUS gene (page 13962, column 1, lines 13 - 17). Indeed, in the discussion section, Waterhouse et al. openly admit that while several models have been proposed for the induction and operation of PTGS, none completely fit the observed results (page 13692, column 1, lines 3 - 6). The research article by Waterhouse et al. clearly states that: "in most models, the antisense RNA or cRNA is proposed to hybridize with the target RNA in some way marking it for degradation." (page 13692, column 2, lines 4 - 6). This statement clearly indicates that it is not known how the target RNA is marked or recognized for regulated degradation within the cell. It is not suggested what the actual length of the antisense RNA or cRNA is, or could be, let alone what element, i.e. which fragment (length) or what particular fragment of antisense RNA or cRNA may be required. In contrast, the present invention provides methods for the efficient identification of species of short length RNAs associated with PTGS, and further, provides methods for their isolation. The import of the instant invention lies in providing a reliable method in which RNA species of a particular length are identified from environments wherein PTGS is operating and from this, the identification, characterization of such RNA's.

Waterhouse et al. further state in column 2 that there are conflicting ideas about the induction of the degradation system and list a number of papers where other authors have reported various findings (page 13692, column 2, lines 6 - 23). Clearly, the scientific community had not at the time of the Waterhouse et al. publication any real consensus view on how PTGS was manifest, and indeed, no suggestion is made regarding isolation of short RNAs that contribute to PTGS in the manner described in the instant application, let alone how to how to identify them.

In summary, there is no description or suggestion of Waterhouse et al providing a method for the identification,

isolation or diagnosis for gene silencing involving SRMs of about 25 nucleotides associated with PTGS.

Wassenegger et al. is a review article setting forth the different hypotheses on PTGS "on (sic) the light of the most recent data." (page 350, column 1, lines 49 - 51). On page 351, column 1, lines 21 - 28, it is noted that certain current models **speculate** that the PTGS phenomenon is based on short antisense RNA molecules that are produced by a host RdRP.

However, in the next sentence it is rightly cautioned that Mezlaff and co-workers reported that the Chs gene could be silenced via an RdRP independent mechanism. It is then stated that Metzlaff et al. had found a 43nt long element of the Chs mRNA 3' untranslated region that was highly complementary to a part of the coding region. As a first observation, it is clear that there is some controversy surrounding the mechanism of PTGS and how it may be manifest.

The Examiner has indicated that the teaching of Wassenegger et al. suggests that the homology of 60 to 130 bp or 10 - 100 bp between an inactivating transgene and the target sequence can lead to PTGS (page 356, column 1). In general, this section is pure speculation. Wassenegger et al. state "several observations indicated that homology of 60 to 130 bp between an inactivating transgene and the target sequence can lead to PTGS."(page 356, column 1, lines 9 - 11). However, in the context of the present invention there is a clear difference between lengths of <u>homology</u>, and lengths of <u>RNA</u>. Although Wassenegger et al. go on to say that "A *similar* size range (10 to 100 nt) has been proposed for RdRP-produced RNA. " (page 356, column 1, lines 11 - 13; emphasis added), this is arguably contradictory. If anything was taught by this it would be that the area of overlap (i.e., 60-100) was the area of interest - this actually teaches away from the present invention.

Furthermore, additional statements made by Wassenegger et al. teach away from the present invention and certainly do not provide one of ordinary skill in the art with a reasonable expectation of success. Specifically, at page 356, column 1, lines 14-15, it is stated:

> " there is no experimental evidence of RdRP-synthesised antisense RNA in plants." (page 356, column 1, lines 14 - 15).

Indeed, Wassenegger et al. further state that while there is an apparent requirement of transgene RNA, this does not mean that a corresponding asRNA (antisense RNA) is produced,..." (page 356, column 1, lines 20 - 22), although the involvement of an asRNA is postulated. However, reading on, it is clear that this type of postulation did not apparently fit the facts in the two known reported examples that described the detection of asRNAs that were specific for genes undergoing transgene-mediated gene silencing. In this case, the asRNAs detected "are certainly the result from direct transcription of the genomic sequences." It is stated by Wassenegger et al. that there was a disproportion between the amounts of the detected asRNAs and the complementary mRNAs and that a putative PTGS mediated by these asRNAs was questioned (page 356, column 2, lines 1 - 9).

It is at this point (page 356, column 2, lines 10 - 12) that Wassenegger et al stated that:

> "further *ingenious* experiments will be needed to puzzle out, how...asRNA can be determined" .

Such ingenious experiments are disclosed in the present application.

Indeed, on the available evidence as reported by Wassenegger et al. it is clear that many postulations as to the mechanisms involved in PTGS have been made but that none of them appeared to explain the manifestation of PTGS satisfactorily.

The final comment by Wassenegger et al. suggests that the finding of the correct sort of SRMs involved in PTGS is problematic and is not as straightforward as the Examiner implies. The Examiner is directed to the last statement made on page 360, wherein Wassenegger et al. conclude that much more effort is needed to detect abRNA, Rt RNA and asRNA. Wassenegger et al. did not provide or offer any reliable method for detecting such RNAs and were clearly lamenting the fact that nobody else had as yet done so. The Wassenegger et al. statement clearly shows that the detection of RNA's, the existence of which was merely speculated, was in any case not a simple task. Indeed, the very fact that the present inventors findings, upon which the present invention is based, were published in the highly respected journal, *Science* (after the filing date of the instant invention) indicates the invention constitutes a significant advance in the art for detecting and identifying RNAs associated with PTGS. These findings had sufficient scientific merit to be accepted for publication within such a very high profile journal. This fact in itself strongly suggests that the science reported by the present inventors made a significant impact within the scientific community and as such, it supports Applicants view that the cited prior art does not render the presently claimed invention obvious.

In summary, several points can be made concerning the teaching of Wassenegger et al. Firstly, it is clear that although the phenomenon of PTGS has been previously observed, the mechanism of how it is manifest is not clearly understood. Secondly, the significance of the 43nt long Chs mRNA is only

addressed in terms of its possible role in PTGS, according to the model proposed by Metzlaff et al. Wassenegger et al. were not concerned with teaching methods for the detection of RNA nor indeed were they concerned with the development of a method in which short RNA's of any size, let alone within the size range presently claimed, could be detected and identified. Indeed, it is quite clear that the Wassenegger et al. teaching laments the lack of an effective method for the detecting of various species of RNA involved in PTGS, including asRNA, and call for more effort to detect RNA species involved in PTGS.

A close reading of the Wassenegger et al. and Waterhouse et al. references leads the skilled artisan into an intellectual cul de sac. Neither publication taken singly or in combination teaches or suggests a reliable method for the identification of RNAs involved in PTGS. In the first mentioned paper, the skilled artisan is prompted to review several hypotheses relating to the phenomenon of PTGS and is forced to conclude that the state of understanding in the art based on these hypotheses or any single hypothesis is not at all settled. The deficiencies in the teaching of Wassenegger et al, are, therefore, not made up by the teaching of Waterhouse et al. Waterhouse et al. do not provide a method for the identification of, isolation of, or detection of gene silencing involving SRMs associated with PTGS, and indeed, provide no suggestion on how to detect SRMs that appear to be involved in PTGS. The combined teaching of Wassenegger et al. and Waterhouse et al. suggest that RNA is somehow involved in PTGS and both teachings postulate theories on how it may be involved and how PTGS may work. However, the teaching of neither journal article taken singly or in combination makes any allusion, specific or implied to the provision of any reliable detection method for SRMs or clearly teaches that SRMs of a particular size are involved in PTGS. Thus, the skilled artisan is left with at

least three dilemmas to solve. One dilemma is to determine what, if any, RNA species are associated with PTGS. Associated with the first dilemma is a second: how to reliably detect such RNA species. Without being able to detect RNA species reliably, an analysis of whether or not such RNA species share features in common is rendered problematic. A third dilemma is to find out what size the RNA fragments may be that are associated with PTGS. The prior art does not teach, allude to, or suggest means to find solutions to any of these dilemmas. In light of the foregoing, Applicants respectfully submit that the Examiner has failed to establish a prima facie case of obviousness based on the combination of Waterhouse et al. and Wassenegger et al. Accordingly, Applicants request that the rejection of claims 1, 2, 5-17, 21, 26-29 and 32 under 35 U.S.C. §103 be withdrawn.

In view of the amendments presented herewith and the foregoing remarks, it is respectfully urged that all of the objections and rejections set forth in the January 19, 2001 Official Action be withdrawn, and that this application be passed to issue and such action is earnestly solicited.Respectfully submitted,

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Enclosures: -Marked up draft of claims indicating where amendments have been made
-Abstract of disclosure on a separate sheet

MARKED UP DRAFT OF AMENDED CLAIMS

1. (Amended) A method of determining the occurrence of target gene silencing in a[n organism] plant, which method comprises the steps of:

(i) obtaining a sample of material from said [organism] plant,

(ii) producing a nucleic acid extract from said sample,

(iii) analyzing said extract such as to determine the presence or absence of short RNA molecules which are [approximately] 21- 25 nucleotides in length (SRMs) in said nucleic extract,(iv) correlating the presence of said SRMs in the extract with the occurrence of said target gene silencing in said [organism]plant.

8. (Amended) A method of detecting the silencing of a target gene in [organism] plant as determined in claim 1, which method further comprises the steps of:

(v) characterizing any SRMs which are present in said extract such as to determine sequence identity or similarity with said target gene, and

(vi) correlating the presence of SRMs in said extract which share sequence identity or similarity with said target gene with the silencing of said target gene in said [organism] plant.

9. (Amended) A method in accordance with claim 8, wherein the silencing of said target gene is the [organism] plant is associated with pathogen derived resistance.

10. A method in accordance with claim 8 wherein the silencing of said target gene is the [organism] plant is associated with modification of a specific trait by co-

suppression of the target gene.

11. (Amended) A method of identifying a <u>silenced</u> <u>target</u> gene in a[n organism] <u>plant</u> in which gene silencing is detected as claimed in claim 8, which method further comprises the steps of:

(vii) preparing a library of genes from said organism, and

(viii) identifying those genes in said library which share sequence identity or similarity with any SRMs which are present in the extract as being genes which are silenced in the organism.

14. (Amended) A process according to claim 13 which further comprises the step of transferring the RNA molecules on the gel to a [hybridisation] <u>hybridization</u> membrane by electrophoresis.

17. (Amended) A process for isolating a silencing agent comprising SRMs for a target gene, which process comprises the steps of:

(i) silencing said target gene in said [organism] <u>plant</u>,

(ii) obtaining a sample of material from said [organism] <u>plant</u>,

(iii) performing a process in accordance with claim 16 to isolate said SRMs.

21. (Amended. A method according to claim [19] <u>1</u> wherein the target gene is a plant gene selected from the [list] <u>group</u> consisting of: a ripening specific gene; a gene involved in pollen formation; a gene involved in lignin biosynthesis; a gene involved in flower pigment production; a gene involved in

regulatory pathways controlling development or environmental responses; a gene involved in the production of toxic secondary metabolites.

26. (Amended) A DNA construct in which a promoter is operably linked to DNA for transcription in a host cell to generate a silencing agent for a target gene, said construct encoding a molecule [being] selected from either:

(i) one or more SRMs, or

(ii) an anti-sense RNA molecule capable of targeting a region of said target gene selected in accordance with the method of claim [18] 1.